

THE CLAIMS

What is claimed is:

- Sub
C1
1. A fusion protein exhibiting a phase transition, the fusion protein comprising:
 - (a) one or more biological molecules;
 - (b) one or more proteins exhibiting a phase transition joined to the biologically active molecule; and
 - (c) optionally, a spacer sequence separating any of the protein(s) of (b) from any of the biological molecule(s) of (a).
 2. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises a component selected from the group consisting of peptides, non-peptide proteins, lipids, and carbohydrates.
 3. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises a peptide.
 4. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises a biologically active protein.
 5. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises a therapeutic protein.
 6. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises an enzyme useful in industrial biocatalysis.
 7. The fusion protein of claim 1 wherein the biological molecule of 1(a) comprises an antibody or antibody fragment.

Sub
C2

8. The fusion protein of claim 7 wherein the antibody or antibody fragment has affinity for a protein of interest, and wherein upon binding to the protein of interest, the fusion protein retains some or all of its phase transition character.

9. The fusion protein of claim 1 wherein the phase transition is mediated by one or more means selected from the group comprising:

- (a) changing temperature;
- (b) changing pH;
- (c) addition of organic solutes and/or solvents,
- (d) side-chain ionization or chemical modification; and
- (e) changing pressure.

10. The fusion protein of claim 1 wherein the phase transition is mediated by means comprising raising temperature.

11. The fusion protein of claim 1 wherein the one or more protein(s) of 1(b) comprises protein exhibiting a β -turn.

12. The fusion protein of claim 1 wherein the one or more protein(s) of 1(b) comprises oligomeric repeats of the pentapeptide Val-Pro-Gly-X-Gly, wherein X is any natural or non-natural amino acid residue, and wherein X optionally varies among oligomeric repeats.

13. The fusion protein of claim 12 wherein the X component(s) of the oligomeric repeats comprise(s) a naturally-occurring amino acid residue.

14. The fusion protein of claim 12 wherein the X component(s) of the oligomeric repeats comprise(s) a non-naturally-occurring amino acid residue.

15. The fusion protein of claim 12 wherein the X component(s) of the oligomeric repeats comprise(s) one or more amino acid residues selected from the group consisting of: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine residues.
16. The fusion protein of claim 12 wherein any two or more of the oligomeric repeats are separated by one or more amino acid residues which do not eliminate the phase transition characteristic of the fusion protein.
17. The fusion protein of claim 12 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the ELP is greater than about 75%.
18. The fusion protein of claim 12 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the ELP is greater than about 85%.
19. The fusion protein of claim 12 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the ELP is greater than about 95%.
20. The fusion protein of claim 1 comprising the spacer sequence of 1(c).
21. ~~The fusion protein of claim 20 wherein the spacer sequence comprises a proteolytic cleavage site.~~
22. The fusion protein of claim 1 wherein the fusion protein further comprises a signal peptide.
23. The fusion protein of claim 22 wherein the signal peptide is cleavable from the fusion protein by enzymatic cleavage.
24. The fusion protein of claim 22 wherein the signal peptide directs secretion of the fusion protein from the cell.

25. The fusion protein of claim 1 wherein the fusion protein or any of the biological molecule(s) of 1(a), protein(s) of 1(b), and spacer sequence of 1(c) (when present) is recombinantly produced.

26. The fusion protein of claim 1 wherein the fusion protein or any of the biological molecule(s) of 1(a), protein(s) of 1(b), and spacer sequence of 1(c) (when present) is synthetically produced.

27. A fusion protein exhibiting a phase transition, the fusion protein comprising:

- (a) one or more protein(s) of interest;
- (b) one or more protein(s) exhibiting a phase transition joined at a C- and/or N-terminus of a protein of (a); and
- (c) optionally, a spacer sequence separating the any of the protein(s) of (a) and/or (b).

28. The fusion protein of claim 27 wherein the phase transition is mediated by means comprising raising temperature.

29. A fusion protein exhibiting a phase transition, said fusion protein comprising:

- (a) one or more proteins of interest;
- (b) one or more β -turn protein(s) joined at a C- and/or N-terminus of any of the proteins of (a); and
- (c) optionally, a spacer sequence separating any of the protein(s) of (a) and/or (b).

30. The fusion protein of claim 29 wherein the phase transition is mediated by means comprising raising temperature.

31. A fusion protein exhibiting a phase transition, the fusion protein comprising:

- (a) a protein of interest;
- (b) a protein exhibiting a phase transition joined at a C- and/or N-terminus of the protein of interest; and
- (c) optionally, a spacer sequence separating the protein of (a) from the protein of (c).
32. The fusion protein of claim 31 wherein the phase transition is mediated by raising temperature.
33. A fusion protein exhibiting a phase transition, said fusion protein comprising:
- (a) a protein of interest;
- (b) a protein exhibiting a β -turn joined at a C- and/or N-terminus of the protein of (a); and
- (c) optionally, a spacer sequence separating the protein of (a) from the protein of (c).
34. The fusion protein of claim 33 wherein the phase transition is mediated by raising temperature.
35. A polynucleotide comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, said fusion protein comprising:
- (a) one or more protein(s) of interest;
- (b) one or more β -turn proteins exhibiting a phase transition joined at a C- and/or N-terminus of (a); and
- (c) optionally, a spacer sequence separating any of the protein(s) of (a) and/or (b).
36. The polynucleotide of claim 35 wherein the phase transition of the fusion protein is mediated by raising temperature.

37. The polynucleotide of claim 35 wherein the protein(s) of 35(a) comprise a biologically active protein.
38. The polynucleotide of claim 35 wherein the protein(s) of 35(a) comprise a therapeutic protein.
39. The polynucleotide of claim 35 wherein the protein(s) of 35(a) comprise an enzyme useful in industrial biocatalysis.
40. The polynucleotide of claim 35 wherein the protein(s) of 35(a) comprise an antibody or antibody fragment.
41. The polynucleotide of claim 40 wherein the antibody or antibody fragment has affinity for a protein of interest, and wherein upon binding to the protein of interest, the fusion protein retains some or all of its phase transition character.
42. The polynucleotide of claim 35 wherein the proteins(s) of 35(b) comprise a β -turn structure.
43. The polynucleotide of claim 35 wherein the proteins(s) of 35(b) comprise an amino acid sequence comprising oligomeric repeats of the pentapeptide Val-Pro-Gly-X-Gly, wherein X is any natural or non-natural amino acid residue, and wherein X optionally varies among oligomeric repeats.
44. The polynucleotide of claim 43 wherein the X component(s) of the oligomeric repeats comprise(s) a naturally-occurring amino acid.
45. The polynucleotide of claim 43 wherein the X component(s) of the oligomeric repeats comprise(s) a non-naturally-occurring amino acid.
46. The polynucleotide of claim 43 wherein the X component(s) of the oligomeric repeats comprise(s) one or more amino acid residues selected from the group consisting of: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine,

histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine residues.

47. The polynucleotide of claim 43 wherein any two or more of the oligomeric repeats are separated by one or more amino acid residues which do not eliminate the phase transition characteristic of the fusion protein.
48. The polynucleotide of claim 47 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the ELP is greater than about 75%.
49. The polynucleotide of claim 47 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the ELP is greater than about 85%.
50. The polynucleotide of claim 47 wherein the ratio of Val-Pro-Gly-X-Gly oligomeric repeats to other amino acid residues of the ELP is greater than about 95%.
51. The polynucleotide of claim 35 comprising the spacer sequence of 35(c).
52. The polynucleotide of claim 51 wherein the spacer sequence comprises a proteolytic cleavage site.
53. The polynucleotide of claim 35 wherein the fusion protein further comprises a signal peptide.
54. The polynucleotide of claim 53 wherein the signal peptide is cleavable from the fusion protein by enzymatic cleavage.
55. The polynucleotide of claim 53 wherein the signal peptide directs secretion of the fusion protein from the cell.
56. An expression vector comprising the polynucleotide of claim 35.
57. A host cell transformed by the expression vector of claim 56, which host cell expresses the fusion protein.

58. A method of producing one or more fusion proteins comprising:
- (a) transforming a host cell with the expression vector of claim 56; and
 - (b) causing the host cell to express the fusion protein.
59. The method of claim 58 wherein the expressed fusion protein comprises a signal sequence directing secretion of the fusion protein from the cell.
60. A method for isolating one or more fusion proteins comprising:
- (a) expressing the fusion protein(s) according to the method of claim 58;
 - (b) disrupting the cells to release the fusion proteins; and
 - (c) isolating the proteins by a method comprising raising temperature.
61. A method for isolating one or more fusion proteins comprising:
- (a) expressing the fusion proteins according to the method of claim 59;
 - (b) isolating the proteins by a method that comprises raising temperature.
62. A method of optimizing size of an ELP expression tag incorporated in a polynucleotide comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, wherein the fusion protein comprises a protein of interest, said method comprising the steps of (i) forming a multiplicity of polynucleotides comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, wherein each of said multiplicity of polynucleotides includes a different-sized ELP expression tag, (ii) expressing corresponding fusion proteins from said multiplicity of polynucleotides, (iii) determining a yield of the desired protein for each of said corresponding fusion proteins, (iv) determining size of particulates for each of said corresponding fusion proteins in solution as temperature is raised above T_i , and (v) selecting an optimized size ELP expression tag according to predetermined selection criteria for maximum recoverable protein of interest from among said multiplicity of polynucleotides.

63. A method of purification of fusion proteins to yield a protein of interest, comprising forming a polynucleotide comprising a nucleotide sequence encoding a fusion protein exhibiting a phase transition, expressing the fusion protein in culture, and subjecting a fusion protein-containing material from said culture to processing involving centrifugation and inverse transition cycling to recover said protein of interest.
64. The method of claim 63, comprising expressing the fusion protein in culture in a well of a microplate.
65. The method of claim 63, comprising processing the fusion protein-containing material from said culture in a well of a microplate.

add B2

add
C9